

WHAT IS CLAIMED IS:

1. A method for identifying a plant disease resistance gene with a specified characteristic, the method comprising:
 - (a) providing a plurality of disease resistance (R) gene segments;
 - 5 (b) recombining the plurality of R gene segments, thereby producing a library of recombinant R gene segments;
 - (c) optionally repeating the recombination of steps (a) and (b) one or more times;
 - (d) expressing at least one recombinant R gene segment in at least one plant cell, and exposing the at least one plant cell to an elicitor of a plant defense response; and
 - 10 (e) detecting at least one plant defense response, thereby identifying a plant disease resistance (R) gene with a specified characteristic.
2. The method of claim 1, further comprising repeating the recombination and screening process of steps (a) through (e) at least one additional time.
3. The method of claim 1, the R gene segments comprising at least one
15 nucleic acid sequence selected from among a disease resistance gene of tomato, rice, Arabidopsis, barley, corn, soybean, flax, sugar beet and wheat.
4. The method of claim 1, the R gene segments comprising at least one nucleic acid sequence selected from among homologs of Bs2, Cf2, Cf4, Cf5, Cf9, Dm3, Fen, Hcr2, Hcr9, Hs1^{pro-1}, I2, L6, LRK10, M, Mlo, Mi, N, Pib, PRF, Pti1, Pto, Rp1-D,
20 RPM1, RPP, RPS2, RPS4, Rx, Xa1 and Xa21.
5. The method of claim 1, further comprising mutating one or more of the segments provided in (a).
6. The method of claim 1, comprising recombining the population of R gene segments in vivo, in vitro or in silico.
- 25 7. The method of claim 6, comprising recombining RNA viruses comprising R gene segments in vivo.

8. The method of claim 7, comprising recombining RNA viruses comprising R gene segments in plant cells.

5 9. The method of claim 1, wherein expressing the at least one recombinant R gene segment comprises stably integrating the at least one recombinant R gene operably linked to a promoter functional in a plant cell into the genome of the at least one plant cell.

10. The method of claim 1, wherein expressing the at least one recombinant R gene segment comprises inoculating the at least one plant cell with a non-integrating viral vector comprising the at least one recombinant R gene.

10 11. The method of claim 10, wherein the non-integrating viral vectors comprise (+) strand RNA viruses, (-) strand RNA viruses, ambisense viruses, single stranded DNA viruses or double stranded DNA viruses.

12. The method of claim 11, wherein the non-integrating viral vector is selected from a tobamovirus, a potexvirus, a potyvirus, a tobravirus or a geminivirus.

15 13. The method of claim 11, wherein expression of the R genes is regulated by at least one viral or non-viral promoter active in the plant cell.

14. The method of claim 13, wherein the promoter is a viral subgenomic promoter.

20 15. The method of claim 1, wherein expressing the at least one recombinant R gene segment comprises infecting the at least one plant cell with a plant pathogen comprising the at least one recombinant R gene.

16. The method of claim 15, wherein the plant pathogen is a bacterial plant pathogen.

25 17. The method of claim 16, wherein the bacterial plant pathogen is a species of *Pseudomonas*.

18. The method of claim 15, wherein the R gene segment further comprises a targeting signal.

19. The method of claim 17, wherein the target signal comprises an AvrBs2 or an AvrPto target signal.

20. The method of claim 1, comprising exposing the at least one plant cell to an elicitor of a plant defense response comprising a product of an Avr gene or Avr gene homolog.

21. The method of claim 20, comprising exposing the at least one plant cell to an Avr gene product produced by a plant pathogen.

22. The method of claim 21, wherein the Avr gene product produced by the plant pathogen is a heterologous Avr gene product.

23. The method of claim 20, comprising exposing the at least one plant cell to an Avr gene product produced by a non-pathogenic microorganism or virus.

24. The method of claim 23, wherein the virus is a non-integrating viral vector.

25. The method of claim 20, comprising exposing the at least one plant cell to an Avr gene product produced by the plant cell.

26. The method of claim 25, wherein the plant cell is a transgenic plant cell expressing an Avr gene.

27. The method of claim 1, comprising detecting at least one plant defense response comprising a hypersensitive (HR) response, a systemic acquired resistance (SAR) response, an induction of genes associated with a HR or a SAR, an accumulation of gene products or compounds associated with a HR or a SAR or a resistance to an infection by a plant pathogen.

28. The method of claim 28, comprising detecting resistance to an infection by a plant pathogen comprises detecting a decrease in symptoms or a decrease in pathogen growth.

29. The method of claim 27, wherein the plant pathogen is a bacterial, fungal, insect or nematode pathogen.

30. The method of claim 27, comprising detecting a plant defense response by one or more of viability staining, visualization of local lesions, measuring calcium flux or monitoring electrolyte leakage.

31. The method of claim 1, wherein the specified characteristic is selected from among ligand binding, downstream signalling and kinase activation.

32. The method of claim 1, further comprising recovering at least one R gene with a specified characteristic.

33. The method of claim 32, comprising recovering the at least one R gene by at least one of PCR, LCR, Q β amplification, cloning, isolation of an RNA transcript and reverse transcription.

34. The method of claim 33, wherein the RNA transcript is a viral RNA transcript.

35. The method of claim 32, further comprising integrating the at least one R gene with a specified characteristic operably linked to a promoter functional in a plant cell into the genome of a plant cell.

36. The method of claim 35, further comprising regenerating the plant cell, thereby producing a transgenic plant that expresses a product of the R gene with a specified characteristic.

37. The method of claim 36, further comprising exposing the transgenic plant to at least one elicitor.

38. The method of claim 37, wherein the elicitor is the product of a recursively recombined Avr gene or Avr gene homolog, or a recursively recombined gene encoding an enzyme catalyzing production of an elicitor.

39. The method of claim 37, detecting at least one plant defense response, thereby identifying an elicitor with a desired property.

40. The method of claim 39, wherein the desired property is interacting with the product of the R gene with a specified characteristic.

41. A transgenic plant produced by the method of claim 36.

42. A method of conferring resistance to at least one plant pathogen by introducing the R gene with a specified characteristic of claim 32 into a plant or plant cell.

5 43. The method of claim 42, comprising introducing the R gene by inoculating the plant or plant cell with a non-integrating viral vector comprising the R gene with a specified characteristic.

10 44. The method of claim 42, comprising stably integrating the R gene with a specified characteristic operably linked to a promoter functional in a plant into a plant cell, and regenerating the plant cell comprising the R gene with a specified characteristic into a transgenic plant.

45. A method for identifying an elicitor of a plant defense response with a desired property, the method comprising:

- 15 (a) providing a plurality of nucleic acid segments comprising at least one elicitor or enzyme catalyzing production of an elicitor of a plant disease response;
- (b) recombining the plurality of nucleic acid segments, thereby producing a library of recombinant nucleic acids encoding elicitors or enzymes catalyzing production of elicitors;
- 20 (c) optionally repeating the recombination of steps (a) and (b) one or more times;
- (d) exposing at least one plant cell to at least one elicitor encoded by or produced by an enzyme encoded by a member of the library of recombinant nucleic acids of step (b); and
- (e) detecting at least one plant defense response, thereby identifying at least one elicitor with a desired property.

25 46. The method of claim 45, further comprising repeating the recombination and screening process of steps (a) through (e) at least one additional time.

47. The method of claim 45, the plurality of nucleic acid segments of step (a) comprising at least one nucleic acid sequence comprising a viral nucleic acid sequence, a bacterial nucleic acid sequence, a fungal nucleic acid sequence, an insect nucleic acid or a nematode nucleic acid.

48. The method of claim 45, the plurality of nucleic acid segments of step (a) comprising at least one nucleic acid sequence selected from an Avr gene or Avr gene homolog.

5 49. The method of claim 45, comprising recombining the plurality of nucleic acids in vivo, in vitro or in silico.

50. The method of claim 49, comprising recombining RNA viruses comprising at least one elicitor or enzyme catalyzing production of an elicitor of a plant disease response in vivo.

10 51. The method of claim 50, comprising recombining RNA viruses comprising at least one elicitor or enzyme catalyzing production of an elicitor of a plant disease response in plant cells.

52. The method of claim 45, comprising exposing the at least one plant cell to at least one elicitor by externally applying the at least one elicitor to the at least one plant cell.

15 53. The method of claim 45, comprising exposing the at least one plant cell to at least one elicitor by inoculating the at least one plant cell with a non-integrating viral vector comprising a member of the library of recombinant nucleic acids encoding elicitors or enzymes catalyzing production of elicitors.

20 54. The method of claim 53, wherein the non-integrating viral vector comprises (+) strand RNA viruses, (-) strand RNA viruses, ambisense RNA viruses, single stranded DNA viruses or double stranded DNA viruses.

55. The method of claim 54, wherein the non-integrating viral vector is selected from a tobamovirus, a potexvirus, a potyvirus, a tobnavirus or a geminivirus.

25 56. The method of claim 54, wherein expression of the elicitor or enzyme catalyzing an elicitor is regulated by at least one viral or non-viral promoter active in the plant cell.

57. The method of claim 56, wherein the promoter is a viral/subgenomic promoter.

58. The method of claim 45, comprising exposing the at least one plant cell to at least one elicitor by infecting the at least one plant cell with a plant pathogen comprising a member of the library of recombinant nucleic acids encoding elicitors or enzymes catalyzing production of elicitors.

59. The method of claim 58, wherein the plant pathogen is a bacterial plant pathogen.

60. The method of claim 59, wherein the bacterial plant pathogen is a species of *Pseudomonas*.

61. The method of claim 45, wherein the at least one plant cell comprises a cultured plant cell, a plant protoplasts, a plant tissue, an isolated plant organ, an intact plant organ or a whole plant.

62. The method of claim 61, wherein the at least one plant cell expresses an R gene with a specified characteristic.

63. The method of claim 62, wherein the at least one plant cell comprises a transgenic plant cell.

64. The method of claim 63, wherein the R gene with a specified characteristic is a recursively recombined R gene.

65. The method of claim 45, comprising detecting a plant defense response selected from among a plant disease response, a hypersensitive (HR) response, and a systemic acquired resistance (SAR) response, induction of a gene associated with a HR or SAR, an accumulation of gene products or compounds associated with a HR or SAR, or a resistance to an infection.

66. The method of claim 45, comprising detecting a plant defense response by one or more of viability staining, visualization of local lesions, measuring calcium flux or monitoring electrolyte leakage.

67. The method of claim 45, wherein the desired property is selected from among binding properties, response elicitation.

68. The method of claim 45, further comprising recovering at least one nucleic acid encoding an elicitor with a desired property or an enzyme catalyzing production of an elicitor with a desired property.

69. The method of claim 68, comprising recovering the at least one nucleic acid by at least one of PCR, LCR, Q β amplification, cloning, isolation of an RNA transcript and reverse transcription.

70. The method of claim 69, wherein the RNA transcript is a viral RNA transcript.

71. A method of inducing a plant defense response by exposing at least one plant cell to the elicitor with a desired property of claim 45.

72. The method of claim 71, comprising inoculating the at least one plant cell with a non-integrating viral vector comprising a nucleic acid encoding the elicitor with a desired property.

73. A method for identifying a functional interaction between a plant disease resistance gene and an elicitor, the method comprising:
(i) introducing a first viral vector comprising a plant disease resistance (R) gene, and a second viral vector comprising a gene encoding an elicitor or enzyme catalyzing production of an elicitor into at least one plant cell, such that the R gene and the elicitor are cytoplasmically expressed in the at least one plant cell; and
(ii) detecting at least one plant defense response, thereby identifying a functional interaction between the R gene and the elicitor.

74. The method of claim 1, wherein the viral vectors comprise non-integrating viral vectors selected from among (+) strand RNA viruses, (-) strand RNA viruses, ambisense RNA viruses, single stranded DNA viruses and double stranded DNA viruses.

75. The method of claim 74, wherein the non-integrating viral vector is selected from a tobamovirus, a potexvirus, a potyvirus, a tobnavirus or a geminivirus.

76. The method of claim 74, wherein expression of the R genes is regulated by at least one viral or non-viral promoter active in the plant cell.

5 77. The method of claim 76, wherein the promoter is a viral subgenomic promoter.

78. The method of claim 73, wherein at least one of the R gene or the gene encoding an elicitor or enzyme catalyzing production of an elicitor is a member of a library of genes or gene segments, which library comprises one or more of a genomic
10 library, an expression library, a transcript library, a DNA library, an RNA library, a PCR amplicon library, an EST library, a mutant library and a recursively recombined library.

79. The method of claim 73, wherein at least one of the R gene or the gene encoding an elicitor or enzyme catalyzing production of an elicitor comprise recursively recombined genes.

15 80. The method of claim 73, wherein the population of plant cells comprises cultured plant cells, plant protoplasts, plant tissues, isolated plant organs, intact plant organs or whole plants.

81. The method of claim 73, comprising detecting a plant defense response selected from among a plant disease response, a hypersensitive (HR) response, a
20 systemic acquired resistance (SAR) response, an induction of genes associated with a HR or SAR response, an accumulation of gene products or compounds associated with a HR or SAR response, a resistance to infection by a plant pathogen, a decrease in symptoms of an infection, and a reduction in pathogen growth.

82. The method of claim 73, comprising detecting a plant defense
25 response by one or more of viability staining, visualization of local lesions, measuring calcium flux or monitoring electrolyte leakage.

83. A method for identifying a functional interaction between a plant disease resistance gene and an elicitor, the method comprising:

- (i) exposing at least one plant cell to a plant pathogen comprising an elicitor of a plant defense response and a plant disease resistance (R) gene; and
(ii) detecting at least one plant defense response, thereby identifying a functional interaction between the R gene and the elicitor.

5 **84.** The method of claim 83, wherein the plant pathogen comprises a bacterial plant pathogen.

85. The method of claim 84, wherein the bacterial plant pathogen is a species of *Pseudomonas*.

10 **86.** The method of claim 83, wherein the plant disease resistance (R) gene is a member of a library of genes or gene segments, which library comprises one or more of a genomic library, an expression library, a DNA library, A PCR amplicon library, an EST library, a mutant library and a recursively recombined library.

87. The method of claim 83, wherein a product of the R gene is translocated from the pathogen to the plant cell by a secretory system of the pathogen.

15 **88.** The method of claim 87, wherein the secretory system of the pathogen comprises a Type III secretory system.

89. The method of claim 83, wherein the R gene segment further comprises a targeting signal.

20 **90.** The method of claim 87, wherein the target signal comprises an AvrBs2 or an AvrPto target signal.

91. The method of claim 83, wherein the population of plant cells comprises cultured plant cells, plant protoplasts, plant tissues, isolated plant organs, intact plant organs or whole plants.

25 **92.** The method of claim 83, comprising detecting a plant defense response selected from among a plant disease response, a hypersensitive (HR) response, a systemic acquired resistance (SAR) response, an induction of genes associated with a HR or SAR response, an accumulation of gene products or compounds associated with a HR

or SAR response, a resistance to infection by a plant pathogen, a decrease in symptoms of an infection, and a reduction in pathogen growth.

5 **93.** The method of claim 83, comprising detecting a plant defense response by one or more of viability staining, visualization of local lesions, measuring calcium flux or monitoring electrolyte leakage.

Sub 82
94. A bio-detector comprising:
(i) an R gene encoding a product capable of activation by at least one elicitor; and
(ii) a reporter operably linked to a promoter responsive to the activated product of the R gene.

SUB A1 10 **95.** The bio-detector of claim 83, wherein the R gene comprises a recursively recombined R gene with a specified characteristic.

Sub 82
96. The bio-detector of claim 95, wherein the R gene encodes a product capable of activation by a designated elicitor.

15 **97.** The bio-detector of claim 96, wherein the designated elicitor is an Avr gene product.

98. The bio-detector of claim 83, wherein the reporter comprises a green fluorescent protein (GFP), a carotenoid biosynthetic enzyme, an anthocyanin regulatory gene or a luciferase.

SUB A2 20 **99.** The bio-detector of claim 83, wherein the promoter comprises a promoter derived from a gene in a systemic acquired resistance (SAR) pathway.

100. The bio-detector of claim 83, wherein the promoter comprises a PR promoter.

101. A plant or plant cell comprising the bio-detector of claim 83.

Sub 82
25 **102.** The plant or plant cell of claim 101, wherein one or more component of the bio-detector is stably integrated into a chromosome.

103. The plant or plant cell of claim 101, wherein one or more component of the bio-detector is extrachromosomally replicated.

104. The plant or plant cell of claim 103, wherein the one or more extrachromosomally replicated component of the bio-detector comprises a non-integrating viral vector.

105. A method for producing a gene with a desired property, the method comprising:

(a) introducing a plurality of RNA viral vectors comprising one or more gene of interest into at least one cell;

(b) growing the cell under conditions permitting cytoplasmic recombination between the plurality of RNA viral vectors, thereby producing a library of recombinant RNA viral vectors;

(c) optionally recovering at least one recombinant viral vector and repeating steps (a) and (b);

(d) identifying at least one RNA viral vector comprising a gene with a desired property.

106. The method of claim 105, comprising introducing the plurality of RNA viral vectors by inoculating at least one cell with infectious viral transcripts.

107. The method of claim 105, comprising introducing the plurality of RNA viral vectors by introducing a plurality of cDNA molecules corresponding to viral transcripts.

108. The method of claim 107, wherein viral transcripts comprising the plurality of cDNA molecules are produced in the cytoplasm of the at least one cell.

109. The method of claim 107, wherein the plurality of cDNA molecules are introduced by electroporation, microinjection, biolistics, agrobacterium mediated transformation or agroinfection.

110. The method of claim 105, wherein the RNA viral vector comprises a plant viral vector.

111. The method of claim 110, wherein the RNA viral vector is selected from among a tobamovirus, a potyvirus, a tobnavirus and a potexvirus.

112. The method of claim 110, wherein the RNA viral vector comprises a Tobacco Mosaic Virus (TMV), a TMV homolog or an engineered viral vector derived
5 from a TMV or TMV homolog.

113. The method of claim 105, wherein the gene of interest comprises a protein coding sequence.

114. The method of claim 105, wherein the at least one cell comprises a plant cell.

10 **115.** The method of claim 114, wherein the plant cell comprises an isolated plant cell, a protoplast, a plant explant, a plant tissue or an intact plant.

116. The method of claim 114, comprising growing the plant cell in suspension culture.

15 **117.** The method of claim 114, comprising growing at least one intact plant comprising the plant cell.

118. The method of claim 105, wherein the cytoplasmic recombination is mediated by template switching of an RNA polymerase expressed by the at least one cell.

119. The method of claim 118, wherein the RNA polymerase is a plant viral RNA polymerase.

20 **120.** The method of claim 118, wherein the RNA polymerase is a mutant or engineered viral RNA polymerase that enhances the frequency of homologous or non-homologous RNA recombination relative to a wild-type plant viral RNA polymerase.

121. The method of claim 120, wherein the mutant or engineered viral RNA polymerase is produced by a directed evolution process.

25 **122.** The method of claim 121, wherein the directed evolution process comprises a DNA or RNA recombination procedure.

123. The method of claim 105, comprising recovering at least one recombinant viral vector by isolating RNA from the at least one cell.

124. The method of claim 105, comprising identifying the at least one RNA viral vector comprising a gene with a desired property by selection or screening.

5 **125.** The method of claim 105, comprising introducing at least a first RNA viral vector incapable of systemic infection in a plant and a second RNA viral vector incapable of systemic infection in a plant, which first and second viral vectors have complementary mutations in genes essential for systemic infection, and identifying at least one recombinant RNA viral vector by selecting or screening for RNA viral vectors capable
10 of systemic infection.

126. The method of claim 125, wherein the genes having complementary mutations comprise one or more of a gene encoding a viral movement protein or a gene encoding a viral coat protein.

127. The method of claim 125, wherein selecting or screening is performed
15 by sampling a plant cell or tissue remote from the site of introduction.

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